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Prolonged depletion of antioxidant capacity following ultra-endurance exercise

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Running head: Ultra-running and glutathione depletion

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Abstract

Purpose: The purpose of this study was to examine the short and long-term (up to 1-month) impact of an ultra-endurance running event on redox-homeostasis. **Methods:** Markers of oxidative stress and antioxidant capacity in peripheral blood were assessed following a single-stage 233 km (143 mile) running event. Samples were collected from nine men (mean age \pm SD; 46.1 ± 5.3 y; body mass index 24.9 ± 2.3 kg·m⁻², maximal-oxygen uptake 56.3 ± 3.3 ml·kg⁻¹·min⁻¹). Peripheral blood mononuclear cells (PBMC) were assayed for non-specific DNA damage (frank strand breaks) and damage to DNA caused specifically by oxidative stress (formamidopyrimidine DNA glycosylase (FPG)-dependent damage). Protein carbonylation (PC) and lipid peroxidation (LPO) were assessed in plasma. Reduced glutathione (GSH) was measured in whole blood. **Results:** PBMC frank strand breaks were elevated above baseline at 24 hours post-race ($p < .001$). FPG-dependent oxidative DNA damage was increased immediately post-race ($p < .05$). PC remained elevated for 7 days following the race ($p < .04$) whereas LPO was increased for 24 hours ($p < .05$) and fell below baseline 28 days later ($p < .05$). GSH, a measure of antioxidant capacity also showed a biphasic response, increasing by one third post-race ($p < .01$) and falling to two-thirds of baseline levels 24 hours later ($p < .001$). GSH remained depleted to approximately one third of pre-race values 28 days post-race ($p < .01$). **Conclusions:** Ultra-endurance exercise causes oxidative stress, which persists for 1 calendar month depending on the specific biomarker examined. These results suggest that ultra-endurance events are associated with a prolonged period of reduced protection against oxidative stress.

Keywords: Glutathione, comet assay, protein carbonylation, lipid peroxidation, ultra-endurance exercise.

Introduction

Paragraph number 1. Research consistently shows that ultra-endurance exercise results in the formation of reactive oxygen species (ROS) (17, 27). The extent of this production has the potential to overwhelm antioxidant defences, causing oxidative stress (13, 27). Oxidative stress is associated with damage to proteins, lipids and DNA (27), which has been causally related to ageing, and the development of cancer and cardiovascular disease (28, 39, 40). Consequently, there has been substantial debate as to whether the health benefits of physical activity extend to more extreme forms of endurance exercise (15, 17). For example, epidemiological studies show that individuals taking part in an exceptionally large amount of physical activity have impaired cardiovascular health (17).

Paragraph number 2. Exercise-induced oxidative stress is likely the result of superoxide ($O_2^{\bullet-}$) production by the electron ‘leak’ in mitochondria, xanthine oxidase in endothelial cells, or from the inflammatory response of activated leukocytes (13, 27). $O_2^{\bullet-}$ dismutates to hydrogen peroxide (H_2O_2) and, in the presence of transition metals, forms highly reactive hydroxyl radicals (OH^{\bullet}) which rapidly oxidise bodily molecules (13). Arguably, the most robust and accurate methods for assessing oxidative stress are the measurement of ROS-induced modifications to plasma proteins or damage to DNA in peripheral blood mononuclear cells (PBMC). (13). These blood markers correlate well with similar measurements in tissues, and therefore provide an indication of whole-body oxidative stress (37).

Paragraph number 3. ROS production during exercise can also result in alterations to antioxidant capacity. Measurement of blood antioxidant defences therefore provides another informative method to assess oxidative stress (13). Conceivably, depletion of these defences

could result in decreased protection against ROS. In contrast, an up-regulation of antioxidant defences is often observed following exercise (13, 27), and it is plausible that this process is one of the mechanisms behind the beneficial adaptations to exercise (13, 27). One of the most abundant antioxidant compounds in blood, as with most other body tissues, is reduced glutathione (GSH) (36). GSH acts as an electron donor for the reductive detoxification of hydrogen peroxide (H_2O_2) into water (H_2O) by the enzyme GSH peroxidase (GPx). Decreased GSH is associated with cardiovascular disease and other non-communicable diseases, potentially due to impaired protection leading to oxidative stress (29). GSH also has other important functions, including regulation of immune function and acting as a co-factor for specific enzymatic reactions (11, 16, 34). Thus, this molecule provides a useful measure of oxidative stress.

Paragraph number 4. Many studies have oxidative stress in the context of ultra-endurance exercise, but few have investigated a very prolonged and continuous bout of ultra-endurance exercise consisting of just one sporting activity (e.g., 31). Several studies have examined ironman triathlons, which involve swimming, cycling and running (e.g., 22). Investigations of this kind therefore assume an equal contribution of effects from each sporting modality. Moreover, it is currently unknown whether oxidative stress persists for more than 19 days following such extreme exercise (22). In general, oxidative stress can last for up to 72 hours post-exercise, depending on the biomarker investigated (19, 22, 31, 35). More prolonged effects are observed when antioxidant capacity is the primary measurement. For example, decreased levels of enzymatic antioxidants have been reported 19 days after an ironman competition (22). Although it appears the oxidative footprint left by ultra-endurance exercise is relatively acute, some data is clearly difficult to interpret due to complications in study design. Collecting samples before and

after multiple days of exercise takes into consideration periods of rest and recovery (e.g., 19).

Likewise, examining just one section of a multi-day event also includes effects caused by

previous days of exercise (e.g., 35). Further studies are therefore required to answer the question

of whether ultra-endurance exercise results in alterations to redox-homeostasis which are

detectable one month later.

Paragraph number 5. The present study examined the impact of a single-stage ultra-endurance

running event on redox-homeostasis over a period of 28 days. On the basis of prior research, it

was hypothesised that exercise would cause protein carbonylation, lipid peroxidation and damage

to DNA, which would be accompanied by a reduction in whole blood GSH concentration. It was

anticipated that all parameters would return to baseline levels within 28 days of the race.

Methods

Participants

Paragraph number 6. Nine healthy non-smoking middle-aged men (≥ 40 years) took part in this study (Table 1). Participants abstained from taking vitamin supplements for at least ten weeks prior to the ultra-endurance race. Participants provided informed consent, and the study was approved by the School of Sport and Exercise Sciences research ethics committee, of the University of Birmingham.

[INSERT TABLE 1 HERE]

Baseline measurements

Paragraph number 7. Participants visited the laboratory prior to the ultra-endurance race to have height and body mass recorded, and skin-fold measurements taken for the calculation of percentage body fat (9). Maximal oxygen consumption ($\dot{V}O_2$ max) was directly measured with a graded exercise test on a treadmill adapted from the Bruce protocol (38). Breath-by-breath measurements were recorded every 5 seconds throughout the test (Oxycon Pro, Jaeger, Germany). Heart rate and ratings of perceived exertion (RPE) were recorded during the final minute of each stage. In addition, participants had leisure time physical activity assessed using the international physical activity questionnaire (IPAQ) (5). IPAQ data was expressed as MET-minutes per week, where 1 MET (metabolic equivalent) is equal to resting metabolic rate.

Ultra-endurance race

Paragraph number 8. Participants took part in The Grand Union Canal Race; a single-stage 233 km (145 mile) race from Birmingham to London in the UK. The route comprised mainly gravel or paved tow-paths and bridleways adjacent to the canal. The race started at 06:00 on Saturday the 23rd of May 2009, and participants who completed the race, crossed the finish line between 21:15 the next day, and 01:50 on Monday the 25th of May. Participants undertook the race at their own pace. Diet was not controlled or recorded for logistical reasons and participants consumed food and fluids *ad libitum*. Ambient temperatures during the race were 6-9°C at night and 20-25°C during the day. Relative humidity was 57-64%.

Experimental design

Paragraph number 9. Blood samples were collected from a forearm vein less than 60 minutes before the race and upon completion (mean 65 min \pm 64 SD after). Additional blood draws were taken at 24 hours (mean 26 hours \pm 4 SD after), 7 days, and 28 days post-race. Participants were seated for 5 min prior to all blood draws, and refrained from exercising for at least 7 days following the race.

Blood sample processing

Paragraph number 10. Blood was collected into ethylene-diamine-tetra-acetic acid (EDTA) vacutainer tubes (Becton-Dickinson, Oxford, UK) and immediately stored at 4°C. PBMC and plasma were obtained within 60 min using a portable centrifuge (E8F Portafuge, LW scientific, Georgia, USA). PBMC were isolated using density gradient centrifugation. Briefly, whole blood was diluted 1:1 with Phosphate Buffered Saline (PBS; Invitrogen, Paisley, UK) and carefully layered onto Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden) before centrifuging at 1350 \times g for 30 min. The buffy coat was aspirated, and cells washed in PBS at 1350 \times g for 10 min.

The pellet was re-suspended 1:1 in foetal bovine serum (FBS) 10% dimethyl sulfoxide (DMSO). Plasma was obtained by centrifuging whole blood at $1350 \times g$ for 15 min. PBMC, plasma and whole blood were immediately frozen in multiple aliquots using dry ice, and later transferred to -80°C storage until analysis.

General analytical procedures

Paragraph number 11. Total plasma protein concentration was determined using the bicinchoninic acid method as described by Smith *et al.* (32). Biochemical parameters were expressed relative to protein concentration, cell number, or were corrected for changes in plasma volume according to Dill and Costill (7) as appropriate. Haemoglobin and haematocrit was assessed using a Coulter ACT^{diff} haematology analyser (Beckman-Coulter, High Wycombe, UK). Chemicals were obtained from Sigma-Aldrich, UK, unless otherwise stated.

Reduced glutathione

Paragraph number 12. Reduced glutathione (GSH) was measured in whole blood frozen 1:1 with FBS (10% DMSO) using a commercially available fluorescence-based assay according to manufacturer's instructions (Promega GSH-Glo, Wisconsin, USA).

DNA damage

Paragraph number 13. A non-specific measure of DNA damage, Frank strand breaks to PBMC DNA, were measured using the alkaline single-cell gel electrophoresis (comet) assay (30). DNA damage caused by oxidative stress was assessed using *Escherichia coli* Formamidopyrimidine DNA glycosylase (FPG) digestion for the detection of oxidised purine lesions (FPG-dependent oxidative DNA damage) (4). Briefly, samples and controls were re-suspended in 1 ml of

Dulbecco's modified Eagle medium (DMEM). Control samples (approximately 4×10^5 cells) were treated with 100 μ M hydrogen peroxide or DMEM for 1 hour at 37°C. Samples and controls were centrifuged at $200 \times g$ for 5 min. Pellets were re-suspended and mixed in 25 μ l DMEM and 300 μ l low-melting point agar. 150 μ l of this cell suspension was added to two separate glass slides (pre-coated with a thin layer of normal-melting point agar) and covered with a glass cover-slip. Slides were left for 30 min at 4°C to solidify. Cover slips were removed, and slides added to lysis buffer (2.5 M NaCl, 0.1 M Na₂EDTA, 10 mM Tris base, 1% Sodium *N*-lauryl sarcosinate, 10% DMSO, and 1% Triton-X 100, pH 10.0) for 1 hour at 4°C. Slides were washed (3 \times 5 min) with FPG enzyme buffer (40 mM HEPES, 0.1 M KCl, 0.5 mM Na₂EDTA and 0.2 mg/ml bovine serum albumin, pH 8.0). Parallel slides were treated with either 50 μ l FPG enzyme buffer containing 1 unit of FPG enzyme (Trevigen, Maryland, USA) or 50 μ l FPG enzyme buffer alone. Following treatment, slides were covered with cover-slips and incubated for 60 min at 37°C in a moist box. After incubation, cover-slips were removed, and slides were transferred to a horizontal electrophoresis tank containing electrophoresis buffer (300 mM NaOH and 1 mM Na₂EDTA, pH > 13.0) and left to stand for 20 min before electrophoresis at 32 V, 300 mA for 20 min ($1.8 \text{ V} \cdot \text{cm}^{-1}$). Slides were neutralised by flooding (3 \times 5 min) in neutralisation buffer (0.4 M Tris, pH 7.5), and stained with 50 μ l of 10 \times Sybr Gold (Invitrogen Ltd, Paisley, UK). Slides were stored at 4°C in a moist box, and examined within 24 hours using a fluorescence microscope (20 \times magnification, 515-560 nm excitation and 590 nm barrier filters respectively; Zeis Axiovert 10, Germany). Median percentage of DNA in comet tails was analysed in 100 comets using computer-based image analysis software (Comet Assay IV, Perceptive Instruments, Suffolk, UK).

Protein carbonylation

Paragraph number 14. Plasma protein carbonylation was measured by enzyme-linked-immunosorbent-assay (ELISA) described by(1). Samples and standards (0.5 mg/ml in sodium carbonate buffer; 50 mM, pH 7.4, 50µl) were allowed to bind to 96-well multi-sorb plates for 60min at 37°C in triplicate (Nunc, Fisher Thermo Scientific, UK). 2, 4-dinitrophenylhydrazine (1mM, 50µl) in 2 M hydrochloric acid was added, and plates incubated at room temperature for 60 min. Plates were blocked overnight at 4°C with 0.1% tris-buffered saline-TWEEN-20. Mouse anti-DNP antibody (1:1000, 50µl) was incubated with samples for 120 min at 37°C, followed by a peroxidise-labelled rat anti-mouse antibody (1:5000, 50µl), incubated for 60 min at 37°C. A citrate phosphate-based substrate (0.15 M, pH 5) was added, and plates left to develop in the dark for 30 min at 37°C. The reaction was stopped with 2 M sulphuric acid and plates read at 490 nm. Values were expressed as nmol/mg of protein.

Lipid peroxidation

Paragraph number 15. Lipid peroxides were assayed in plasma using a modification of the method by el-Saadani *et al.* (10). Samples and positive and negative controls (1:1000 hydrogen peroxide and distilled water respectively) were added to 96-well plates in triplicate. Working reagent (0.2 M Potassium Phosphate, 0.12 M Potassium Iodide, 0.15 mM Sodium Azide, 2g/l Triton-X, 0.1 g/l Alkylbenzyldimethylammonium, 10µM Ammonium Molybdate; 100 µl) was added, and plates incubated at room temperature for 30 min. Plates were read at 340 nm. Lipid peroxide concentration was calculated using the Beer-Lambert-Law with an extinction coefficient of 24600. Values were expressed as nmol/ml plasma,

Statistical analysis

Paragraph number 16. Data were inspected for normal distribution using the Kolmogorov Smirnov test. Non-normally distributed data were transformed logarithmically. For the comet assay, measurements of percent (%) tail DNA were determined to assess the extent of DNA damage as recommended by Duez *et al* (8). Responses to exercise were examined with repeated-measures Analyses of Variance (ANOVAs). Differences compared to baseline were examined using post-hoc paired samples *t*-tests. Statistical significance was accepted at the $p < .05$ level. Effect sizes are presented as η^2 . Data are presented as means \pm standard error of the mean (SEM) unless otherwise stated. Data were analysed using SPSS statistical package version 18.0 for Windows (SPSS Inc, USA).

Results

Paragraph number 17. Four men completed the ultra-endurance race (finishers) and five retired prematurely due to exhaustion (non-finishers). There were no significant physiological differences between finishers and non-finishers, nor were there differences in any of the biochemical or immunological parameters investigated between the two groups (data not shown). Running duration (hours), distance (km) or finishing status (i.e., finisher or non-finisher) did not confound any of the results reported below, as determined by analysis of covariance (14). On this basis, and the fact that all non-finishers had undertaken exercise for more than 12 hours (hh:mm; mean 20:44 \pm 07:15 SD), covering the distance of approximately three marathons (mean 126.7 km \pm 29.7 SD), all participants were included in analyses.

DNA damage in PBMC

Paragraph number 18. Figure 1 shows frank strand breaks and FPG-dependent oxidative DNA damage before and after ultra-endurance exercise. Frank strand breaks increased with exercise (main effects of time; $F_{(4, 32)} = 11.7, p < .001, \eta^2 = .594$) and were elevated above baseline immediately and 24 hours after the race (paired samples t -tests; $t_{(8)} < -5.2, p < .01$, see Figure 1). An increase in FPG-dependent oxidative DNA damage was also observed (main effects of time; $F_{(4, 32)} = 2.8, p < .05, \eta^2 = .257$) and this was elevated above baseline immediately post-race (paired samples t -test; $t_{(8)} = -3.1, p < .05$, see Figure 1).

[INSERT FIGURE 1 HERE]

Whole blood GSH

Paragraph number 19. A biphasic antioxidant response was observed with ultra-endurance exercise (main effects of time; $F_{(4, 32)} = 26.0, p < .001, \eta^2 = .764$, see Figure 2) which reflected the elevated markers of oxidative stress. GSH concentration increased immediately post-race (+32%; paired samples t -test; $t_{(8)} = -3.7, p < .01$) but was depleted to nearly two-thirds (-60%) of baseline levels 24 hours later (paired samples t -test; $t_{(8)} = 6.1, p < .001$). At 7 days and 28 days post-race, GSH remained at approximately half to one third (-45 to -31%) of baseline levels respectively (paired samples t -tests; $t_{(8)} > 3.5, p < .01$, see Figure 2).

[INSERT FIGURE 2 HERE]

Plasma protein carbonylation

Paragraph number 20. Ultra-endurance exercise resulted in significant plasma protein carbonylation (main effects of time; $F_{(4, 32)} = 7.6, p < .001, \eta^2 = .370$) (see Figure 3). Post-hoc comparisons to baseline showed that plasma protein carbonyl concentration was elevated for 7 days after the race (paired samples t -tests; $t_{(8)} > -2.5, p < .04$, see Figure 3).

[INSERT FIGURE 3 HERE]

Plasma lipid peroxidation

Paragraph number 21. Ultra-endurance exercise resulted in plasma lipid peroxidation (main effects of time; $F_{(4, 32)} = 14.0, p < .001, \eta^2 = .636$) (see Figure 4). Plasma lipid peroxide concentration was elevated immediately post-race, and remained at this level for 24 hours, before falling below baseline 28 days later (paired samples t -tests; $t_{(8)} < 3.3, p < .05$, see Figure 4).

[INSERT FIGURE 4 HERE]

Discussion

Paragraph number 22. Studies have shown that ultra-endurance exercise results in oxidative stress, which is alleviated in a matter of hours or days depending on the biomarker assessed (19, 22, 31, 35). The present investigation expands upon previous investigations by employing a more prolonged blood sampling regimen (up to 28 days post-race). This study measured several oxidative stress biomarkers, including a comparison between non-specific DNA damage (i.e., frank strand breaks) and oxidative-specific DNA damage (FPG-dependent oxidative DNA damage).

Paragraph number 23. The results confirmed that ultra-endurance exercise is associated with increases in markers of oxidative stress. FPG-dependent oxidative DNA damage was increased immediately post-race, whereas frank strand breaks and lipid peroxidation were elevated for 24 hours. Augmented protein oxidation was detected 7 days after the race. In addition, we showed for the first time, that whole blood GSH exhibits a large biphasic response; increasing by one-third post-race, and falling almost two-thirds below normal levels 24 hours later. Strikingly, the antioxidant capacity of blood remained at one-third of initial values 28 days after the race - a similar level of GSH depletion as seen in Alzheimers disease patients (3).

Paragraph number 24. The present study showed that lipids were susceptible to peroxidation for 24 hours after ultra-endurance exercise, which is consistent with previous reports (22, 31). This increase in lipid peroxidation was accompanied by a fall in lipid peroxide concentration 28 days post-race. Increased lipid peroxidation is associated with cardiovascular disease, and some

markers of oxidised lipids (e.g., malondialdehyde) are mutagenic to DNA increasing the risk of cancer (23, 28). Conversely, decreased lipid peroxidation has previously been observed following exercise (12, 18) and it has been suggested that this may be one of the mechanisms behind the atheroprotective effect of physical activity (12, 15). Further, increased protein oxidation was detected for up to 7 days post-race which could partly be attributed to the chemical stability of protein carbonyl groups (6). Protein carbonylation has been associated with altered protein function and receptor interaction; for example carbonylation of the protein moiety of low density lipoprotein is known to increase uptake into blood monocytes (2).

Paragraph number 25. In the present study, ultra-endurance exercise caused an increase in frank strand breaks, which remained elevated for 24 hours. Damage to DNA is linked to increased cancer and cardiovascular disease risk (39), and elevated levels of DNA damage have been found in atherosclerotic plaques (20). Following ultra-endurance exercise, some of this damage was attributable to exercise-induced ROS production, since FPG-dependent oxidative DNA damage was increased post-race, returning to baseline within 24 hours. The transient appearance of FPG-dependent oxidative DNA damage, and the subsequent return to baseline, likely reflects up-regulation of repair mechanisms in which oxidised purine bases (e.g., 8-oxoguanine) are repaired or removed by 8-oxoguanine DNA glycosylase (OGG1) (26). Indeed, OGG1 activity is increased approximately 16-18 hours after a marathon (25) which coincides with the return of FPG-dependent oxidative DNA damage to baseline in this study. It is possible that frank strand breaks did not follow the same pattern of repair, because this includes oxidised pyrimidines which are not repaired by OGG1. Instead, pyrimidine lesions are repaired by endonuclease III homolog (NTH1), the activity of which, is not up-regulated following exercise (25). In addition, these purine lesions would not be detected (and therefore not included in our measure of oxidative

DNA damage) by FPG in our assay. As well as the risks that DNA damage might confer, damage to lymphocytes might inhibit cell-mediated immunity post-exercise, which is in line with reports of increased infection risk following endurance exercise events (24).

Paragraph number 26. Perhaps the most striking finding of this study is that GSH was depleted to one third of baseline levels at 28 days post-race. The baseline GSH concentrations observed in this study were not elevated when compared to other reports in healthy individuals (e.g., 31). GSH is a molecule that is key in cellular redox status regulation (11, 16, 34, 36) and consequences of prolonged GSH depletion may include a compromise in immunity. For example, lower GSH is associated with decreased lymphocyte proliferation and increased viral reactivation. (16, 33, 34)

Paragraph number 27. A limitation of this investigation is that a control group was not included. However, prior studies employing a non-exercise control did not observe differences in oxidative stress during the resting control trial (e.g., 21). A further potential limitation is that only 4 out of 5 subjects completed the race. All subjects were included in analyses because even non-finishers engaged in more than 12 hours of continuous exercise, and covered the distance of more than three marathons. Importantly, there were no physiological, biochemical, or immunological differences between finishers and non-finishers. Thus, as we expected, controlling statistically for finishing status (i.e., finisher *versus*. non-finisher), duration of exercise, or running distance had no effects on the results reported.

Paragraph number 28.

This study confirms that ultra-endurance exercise causes oxidative stress, which persists for 1 calendar month post-exercise, depending on the specific biomarker examined. For the first time, we show that blood antioxidant capacity, as measured by GSH, is significantly depleted below baseline for 28 days post-race. These results suggest that ultra-endurance events are associated with a period of reduced protection against oxidative stress.

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Conflict of interest

None.

References

1. Alamdari DH, Kostidou E, Paletas K, Sarigianni M, Konstas AGP, Karapiperidou A, and Koliakos G. High sensitivity enzyme-linked immunosorbent assay (ELISA) method for measuring protein carbonyl in samples with low amounts of protein. *Free Radical Biology and Medicine*. 2005;39(10):1362-7.
2. Aldred S, and Griffiths HR. Oxidation of protein in human low-density lipoprotein exposed to peroxyl radicals facilitates uptake by monocytes; protection by antioxidants in vitro. *Environmental Toxicology and Pharmacology*. 2004;15(2-3):111-7.
3. Bermejo P, Martin-Aragon S, Benedi J, Susin C, Felici E, Gil P, Ribera JM, and Villar AM. Peripheral levels of glutathione and protein oxidation as markers in the development of Alzheimer's disease from Mild Cognitive Impairment. *Free Radic Res*. 2008;42(2):162-70.
4. Collins AR, Duthie SJ, and Dobson VL. Direct enzymic detection of endogenous oxidative base damage in human lymphocyte DNA. *Carcinogenesis*. 1993;14(9):1733-5.
5. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, and Oja P. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;35(8):1381-95.

6. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, and Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. *Clinica Chimica Acta*. 2003;329(1-2):23-38.
7. Dill DB, and Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology*. 1974;37(2):247-8.
8. Duez P, Dehon G, Kumps A, and Dubois J. Statistics of the Comet assay: a key to discriminate between genotoxic effects. *Mutagenesis*. 2003;18(2):159-66.
9. Durnin JV, and Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr*. 1974;32(1):77-97.
10. el-Saadani M, Esterbauer H, el-Sayed M, Goher M, Nassar AY, and Jurgens G. A spectrophotometric assay for lipid peroxides in serum lipoproteins using a commercially available reagent. *J Lipid Res*. 1989;30(4):627-30.
11. Filomeni G, Rotilio G, and Ciriolo MR. Cell signalling and the glutathione redox system. *Biochem Pharmacol*. 2002;64(5-6):1057-64.
12. Ginsburg GS, Agil A, O'Toole M, Rimm E, Douglas PS, and Rifai N. Effects of a single bout of ultraendurance exercise on lipid levels and susceptibility of lipids to peroxidation in triathletes. *Jama*. 1996;276(3):221-5.
13. Halliwell B, and Gutteridge JMC. *Free Radicals in Biology and Medicine*. 3rd ed. Oxford: Oxford Science Publications; 1999, 105-350 p.
14. Jennings JR, and Stine LA. Salient Method, Design and Analysis Concerns. In: JT Cacioppo, LG Tassinary and GG Berntson editors. *Handbook of Psychophysiology*. Cambridge, UK: Cambridge University Press; 2007, pp. 870-99.
15. Kesaniemi YK, Danforth E, Jr., Jensen MD, Kopelman PG, Lefebvre P, and Reeder BA. Dose-response issues concerning physical activity and health: an evidence-based symposium. *Med Sci Sports Exerc*. 2001;33(6 Suppl):S351-8.
16. Kinscherf R, Fischbach T, Mihm S, Roth S, Hohenhaus-Sievert E, Weiss C, Edler L, Bartsch P, and Droge W. Effect of glutathione depletion and oral N-acetyl-cysteine treatment on CD4+ and CD8+ cells. *FASEB J*. 1994;8(6):448-51.
17. Knez WL, Coombes JS, and Jenkins DG. Ultra-endurance exercise and oxidative damage : implications for cardiovascular health. *Sports Med*. 2006;36(5):429-41.
18. Kretzschmar M, Muller D, Hubscher J, Marin E, and Klinger W. Influence of aging, training and acute physical exercise on plasma glutathione and lipid peroxides in man. *Int J Sports Medicine*. 1991;12(2):218-22.
19. Machefer G, Groussard C, Rannou-Bekono F, Zouhal H, Faure H, Vincent S, Cillard J, and Gratas-Delamarche A. Extreme running competition decreases blood antioxidant defense capacity. *J Am Coll Nutr*. 2004;23(4):358-64.
20. Martinet W, Knaapen MW, De Meyer GR, Herman AG, and Kockx MM. Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. *Circulation*. 2002;106(8):927-32.
21. Mastaloudis A, Leonard SW, and Traber MG. Oxidative stress in athletes during extreme endurance exercise. *Free Radical Biology and Medicine*. 2001;31(7):911-22.
22. Neubauer O, Konig D, Kern N, Nics L, and Wagner KH. No indications of persistent oxidative stress in response to an ironman triathlon. *Med Sci Sports Exerc*. 2008;40(12):2119-28.
23. Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE, and Marnett LJ. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. *J Biol Chem*. 2003;278(33):31426-33.

24. Nieman DC. Upper respiratory tract infections and exercise. *Thorax*. 1995;50(12):1229-31.
25. Radak Z, Apor P, Pucsok J, Berkes I, Ogonovszky H, Pavlik G, Nakamoto H, and Goto S. Marathon running alters the DNA base excision repair in human skeletal muscle. *Life Sci*. 2003;72(14):1627-33.
26. Radak Z, Chung HY, and Goto S. Systemic adaptation to oxidative challenge induced by regular exercise. *Free Radic Biol Med*. 2008;44(2):153-9.
27. Sachdev S, and Davies KJ. Production, detection, and adaptive responses to free radicals in exercise. *Free Radic Biol Med*. 2008;44(2):215-23.
28. Schwedhelm E, Bartling A, Lenzen H, Tsikas D, Maas R, Brummer J, Gutzki FM, Berger J, Frolich JC, and Boger RH. Urinary 8-iso-prostaglandin F2alpha as a risk marker in patients with coronary heart disease: a matched case-control study. *Circulation*. 2004;109(7):843-8.
29. Shimizu H, Kiyohara Y, Kato I, Kitazono T, Tanizaki Y, Kubo M, Ueno H, Ibayashi S, Fujishima M, and Iida M. Relationship between plasma glutathione levels and cardiovascular disease in a defined population: the Hisayama study. *Stroke*. 2004;35(9):2072-7.
30. Singh NP, McCoy MT, Tice RR, and Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res*. 1988;175(1):184-91.
31. Skenderi KP, Tsironi M, Lazaropoulou C, Anastasiou CA, Matalas AL, Kanavaki I, Thalmann M, Goussetis E, Papassotiriou I, and Chrousos GP. Changes in free radical generation and antioxidant capacity during ultramarathon foot race. *Eur J Clin Invest*. 2008;38(3):159-65.
32. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, and Klenk DC. Measurement of protein using bicinchoninic acid. *Analytical Biochemistry*. 1985;150(1):76-85.
33. Staal FJ, Roederer M, and Herzenberg LA. Intracellular thiols regulate activation of nuclear factor kappa B and transcription of human immunodeficiency virus. *Proc Natl Acad Sci U S A*. 1990;87(24):9943-7.
34. Suthanthiran M, Anderson ME, Sharma VK, and Meister A. Glutathione regulates activation-dependent DNA synthesis in highly purified normal human T lymphocytes stimulated via the CD2 and CD3 antigens. *Proc Natl Acad Sci U S A*. 1990;87(9):3343-7.
35. Tauler P, Sureda A, Cases N, Aguiló A, Rodríguez-Marroyo JA, Villa G, Tur JA, and Pons A. Increased lymphocyte antioxidant defences in response to exhaustive exercise do not prevent oxidative damage. *Journal of Nutritional Biochemistry*. 2006;17:665-71.
36. Townsend DM, Tew KD, and Tapiero H. The importance of glutathione in human disease. *Biomed Pharmacother*. 2003;57(3-4):145-55.
37. Veskoukis AS, Nikolaidis MG, Kyparos A, and Kouretas D. Blood reflects tissue oxidative stress depending on biomarker and tissue studied. *Free Radic Biol Med*. 2009;47(10):1371-4.
38. Whaley MH, Brubaker PH, and Otto RM. ACSM's Guidelines for exercise testing and prescription. Baltimore (MD): Lippincott Williams and Wilkins; 2006, p 79.
39. Wu LL, Chiou CC, Chang PY, and Wu JT. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clin Chim Acta*. 2004;339(1-2):1-9.
40. Yeh CC, Lai CY, Hsieh LL, Tang R, Wu FY, and Sung FC. Protein carbonyl levels, glutathione S-transferase polymorphisms and risk of colorectal cancer. *Carcinogenesis*. 2010;31(2):228-33.

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Figure captions

Figure 1. Frank strand breaks to PBMC DNA (closed bars) and FPG-dependent oxidative DNA damage (open bars). Data are Means \pm SEM, expressed as percent (%) DNA in the comet tail. ** $p < .01$, * $p < .05$ compared to baseline (paired samples t -tests).

Figure 2. Whole blood reduced glutathione (GSH) concentration. Data are Means \pm SEM. *** $p < .001$, ** $p < .01$ compared to baseline (paired samples t -tests).

Figure 3. Plasma protein carbonyl concentration. Data are Means \pm SEM. *** $p < .001$, ** $p < .01$, * $p < .05$ compared to baseline (paired samples t -tests).

Figure 4. Plasma lipid peroxide concentration. Data are Means \pm SEM. * $p < .05$ compared to baseline (paired samples t -tests).

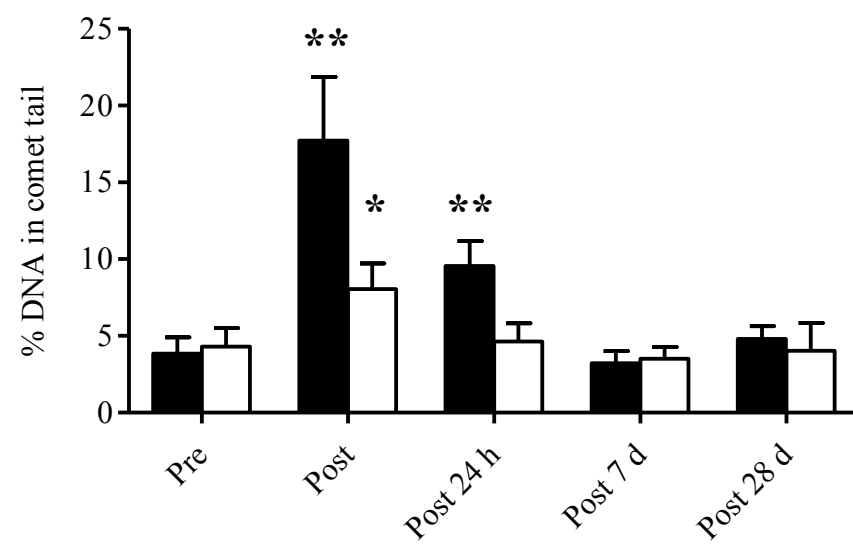
Table 1. Characteristics of participants and results from the ultra-endurance race (mean \pm SD).

Characteristics of participants	<i>n</i> = 9
Age (years)	46.1 \pm 5.3
BMI (kg·m ⁻²)	24.9 \pm 2.3
Body fat (%)	14.1 \pm 2.4
LTPA ^a (Median MET·min·week ⁻¹) ^b	3393 (2040-4850)
Measured $\dot{V}O_2$ max (ml·kg ⁻¹ ·min ⁻¹)	56.3 \pm 3.3
Maximum heart rate at $\dot{V}O_2$ max (bpm)	175 \pm 5
Ultra-endurance race results	
Running distance (km)	174.1 \pm 60.0
Running duration (hh:mm)	30:02 \pm 12:14

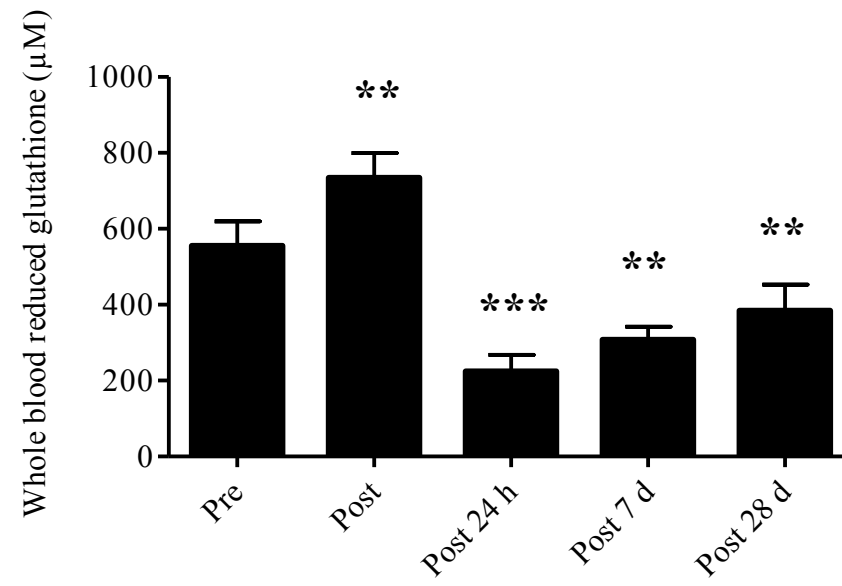
^a Leisure Time Physical Activity, as assessed by the international physical activity questionnaire (IPAQ).

^b Data are medians (min-max).

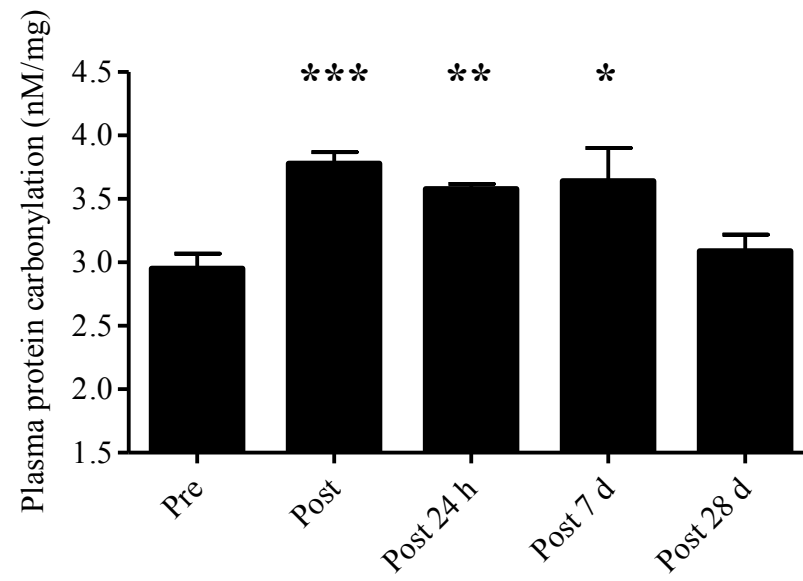
Turner. Ultra-running and glutathione depletion. Figure 1.



Turner. Ultra-running and glutathione depletion. Figure 2.



Turner. Ultra-running and glutathione depletion. Figure 3.



Turner. Ultra-running and glutathione depletion. Figure 4.

